REMARKS/ARGUMENTS

Amendments to the specification. The addition of the paragraph at page 1, line 10, indicates the joint research agreement between the present assignee, Mendel Biotechnology, Inc. and Monsanto Corporation.

The amendments to recite Figures 3A-3B, etc., in the Brief Description of the Drawings have been made in response the Examiner's objection in Item 4 of the Office action.

The amendment to add the file creation date to the description of the Sequence Listing on page 15 of the specification is supported by the file creation date found on the CD-ROM associated with this file.

The remaining amendments to the specification are made to correct obvious typographical errors. The changes of "GALL" and "GAL1" to --GAL4-- are supported by the Li and Herskowitz reference ((1993) *Science* 262: 1870-1874) disclosed in the paragraph in which these changes are being made.

Amendments to the claims. Claims 1-20 were canceled in a previous amendment. Claim 30 is canceled in the present amendment. Previously presented claims 21-29 and 31-40 remain in this application.

Support for the amendment changing "drought" to --water deprivation-- is provided in general, for example, on page 371, line 34, and specifically for G1274, on, for example, page 16, line 25, and on page 410, line 14.

Applicants believe no new matter is added by this amendment.

Office action, item 5, rejection under 35 U.S.C. §112, first paragraph, written description

The rejection for lack of written description is respectfully traversed for the following reasons.

As detailed below and in numerous other references, it is a well-known principle that regulatory (and other) sequences that are closely related, having descended from a common ancestral sequence, often share similar functions. This occurs because entire pathways and associated regulatory machinery co-descend from the common ancestral plant, and a single native pathway component sequence or a closely-related sequence can be plugged into that pathway. Thus, the latter is able to supplant or augment the activity of the native sequence while conferring a similar function. For example, the components of the entire CBF cold-response pathway are highly and coordinately conserved in flowering plants. Constitutive overexpression of *Arabidopsis* CBF transcription factor genes in transgenic *Brassica napus* plants induces expression of orthologs of *Arabidopsis* CBF-targeted genes and increases the freezing tolerance of the plants. Transcripts encoding CBF-like proteins also accumulate rapidly in response to low temperature in distantly-related monocot species (Jaglo et al. (2001) *Plant Physiol.* 127: 910-917).

Thus, by overexpressing one of a set of closely-related sequences in a transgenic plant, the regulatory pathway activity is increased, which in turn amplifies the effects of intermediates and products and ultimately augments the pathway's effects on morphology and physiology. With regard to the sequences encompassed by the present claims, the similar shared functions are to confer to plants similar (that is, G1274-like)

morphological and physiological characteristics, which may be collectively referred to as "G1274 sequelae". The characteristic G1274 (SEQ ID NO: 194) sequelae include the morphological features of flat leaves (observed in lines overexpressing 13 of 20 of the sequences tested), short stature (observed in lines overexpressing 18 of 20 of the sequences tested), bushy appearance (observed in lines overexpressing 17 of 20 of the sequences tested), increased biomass (observed in lines overexpressing 16 of 20 of the sequences tested), and the physiological characteristics of increased water deprivation tolerance (observed in lines overexpressing 14 of 20 of the sequences tested), low nitrogen tolerance (observed in lines overexpressing 13 of 20 of the sequences tested), and cold tolerance (observed in lines overexpressing 11 of 20 of the sequences tested), relative to controls. Many sequences were observed to confer several of these traits, even though they were expressed in a limited number of lines and a non-native species of plant.

While G1274 was the sequence most heavily scrutinized by Applicants in this study, and even though these experiments were performed in non-native species and, in some cases, with just a few lines, all of the other nineteen sequences that are encompassed by the claims and were tested in plants also conferred the characteristic G1274-like morphological and physiological sequelae, thus confirming the related, conserved functions of these sequences and their ability to affect common regulatory pathways.

The Examiner has rejected Claims 21-40 "as containing subject matter that was not described in the specification in such a way as to reasonably convey to one skilled in the art that the inventor(s), at the time the application was filed, had possession of the claimed invention. The Examiner has also stated that "Applicants do not identify essential regions of proteins comprising the conserved domain comprising amino acid coordinates 111-164 of SEQ ID NO: 194", that "Applicants fail to describe a representative number of polynucleotide sequences ... falling within the scope of the claimed genus" and "given the lack of disclosure about other domains that are required along with the conserved domain comprising amino acid coordinates 111-164 of SEQ ID NO: 194, it remains unclear what features identify a protein with the same activity and function as Applicants G1274 of SEQ ID NO: 194".

As far as essential regions and structural features of proteins, conserved domains are well-known in the art as functional and structural features of closely-related proteins; see, for example, Marchler-Bauer et al. (2002) *Nucleic Acids Res.* 30: 281-283, which describes CDD, the "Conserved Domain Database". The NCBI Conserved Domain Database in its own description notes that conserved domains may be used as predictors of evolutionary relationship and function: "[p]roteins often contain several modules or domains, each with a distinct evolutionary origin and function" (currently found at http://www.ncbi.nlm.nih.gov/Structure/cdd/cdd.shtml). To identify conserved domains in a protein sequence, the conserved domain search service also uses a BLAST algorithm very similar to that described in the present specification (paragraph 00223).

Thus, the "essential regions" of G1274 and its closely-related sequences are their conserved domains. Each of the WRKY transcription factor sequences shown in the phylogenetic tree found in Exhibits A and D comprise conserved domains that are at least 55% identical to the conserved domain of G1274. These conserved domains may be found in Exhibits A and B. Conserved domains are conserved because of their importance of retaining the function associated with the domain. The linkage between conserved domains and function is underscored by that fact that gene sequences and encoded polypeptides need not be full length, so long as the desired functional domain of the protein is expressed (Harada, USPN 6,235,975; May 22, 2001). The present specification is replete with descriptions and definitions of conserved domains, including in the specification on page 23, lines 9-12:" [a] 'conserved domain', with respect to presently disclosed polypeptides, refers to a domain within a transcription factor family that exhibits a higher degree of sequence homology, such as at least ... 65% sequence identity including conservative substitutions," and on page 39 at lines 1-2: "[t]ranscription factors that are homologous to the listed sequences will typically share, in at least one conserved domain, at least about 70% amino acid sequence identity". The specification identifies the conserved domain of G1274 as amino acid coordinates 111-164 in Table 5, in the last row on page 82. The specification identifies conserved functions (including DNA binding) associated with conserved domains: '[a]s one of ordinary skill in the art recognizes, transcription factors can be identified by the presence of a region or *domain of structural similarity or identity* to a specific consensus sequence *or the presence of a specific consensus DNA-binding site or DNA-binding site motif*'(page 18, lines 24-26; *emphasis* added) and "a polypeptide fragment can comprise a recognizable structural motif or functional domain such as a DNA-binding site or domain that *binds to a DNA promoter region*, an activation domain, or a domain for protein-protein interactions, and *may initiate transcription* (page 28, lines 33-35; *emphasis* added).

Conserved domains such as conserved DNA binding domains may be used to identify phylogenetically-related sequences that have a minimum (e.g., 70% or more) sequence identity with the similar domain in G1274, that is, domains with a degree of relatedness that may be used as indicators of similar or identical regulatory function. A statement by Hurley et al. (2001) *Trends Biochem. Sci.* 27: 48-53, sums up the state-of-the-art in the understanding that conserved domains are effective indicators and predictors of related protein function: "[o]nce the function of a particular domain from one protein is well understood, powerful and testable inferences can be made as to the function of the many other proteins that contain that domain (Hurley, *supra*, on page 48, paragraph 1, lines 10-14, which describes "hypothesis-driven experiments" for determining related functions of signaling and DNA-binding domains). This is precisely what Applicants did when they identified G1274 as a "member of the WRKY family of transcription factors"(page 409, line 31) and which "have been sometimes categorized by class, family, and sub-family according to their structural content and consensus DNA-binding site motif" (page 19, lines 31-32), identified the conserved domain of G1274 in Table 5 and "[t]ranscription factors that are homologous to

the listed sequences will typically share, in at least one conserved domain, at least about 70% amino acid sequence identity" (page 39, lines 1-2), identified the function of G1274, for example, in Tables 4 and 6 and on page 409, line 29 through page 410, line 31, and identified related sequences having similar conserved domains, some of which have been shown to function similarly to G1274, as described below.

In 2001, Zmasek and Eddy ((2001) *Bioinformatics* 17: 821-828) taught that sequence function prediction performed using methods based only on pairwise sequence similarity "tends to classify novel sequences too aggressively". It is common for groups of genes that are similar in sequence to have diverse (although usually related) functions (Eisen (1998) *Genome Res.* 8: 163-167). An initial analysis of functional relatedness based on sequence similarity alone may not provide one with a means to determine where similarity ends and functional relatedness begins. Fortunately, functional predictions can be greatly improved by focusing on how the genes became similar in sequence (i.e., by evolutionary processes) rather than on the sequence similarity itself (Eisen, *supra*). In fact, many specific examples exist in which gene function has been shown to correlate well with gene phylogeny (Eisen, *supra*). Thus, "[t]he first step in making functional predictions is the generation of a phylogenetic tree representing the evolutionary history of the gene of interest and its homologs. Such trees are distinct from clusters and other means of characterizing sequence similarity because they are inferred by techniques that help convert patterns of similarity into evolutionary relationships .... After the gene tree is inferred, biologically determined functions of the various homologs are overlaid onto the tree. Finally, the structure of the tree and the relative phylogenetic positions of genes of different functions are used to trace the history of functional changes, which is then used to predict functions of [as yet] uncharacterized genes" (Eisen, *supra*).

At the time the present application was filed, Applicants understood that phylogenetically-related sequences, by definition, descend from a common ancestral sequence and that orthologous sequences retain similarity in function. Applicants described on page 121, lines 13-15, how "[a]ny sequence herein can be used to identify a similar, homologous, paralogous, or orthologous sequence in another plant. This provides means for identifying endogenous sequences in other plants that may be useful to alter a trait". Applicants also disclosed that orthologous sequences may be found using a systematic phylogenetic approach on page 36, line 34 through page 37, line 7. As was known in the art and practiced by Applicants, phylogenetic trees may be generated and sequences that fall into specific subtrees (clades or subclades) that are considered orthologous (that is, having similar ancestry and retained function) may be identified. Once orthologs are identified using phylogenetic analysis, it becomes a routine matter to compare the sequences in pair-wise fashion and identify conserved regions or domains and compare relatedness to identity (page 36, lines 20-22 and on page 39, line 25 through page 40, line 25) to determine the threshold of sequence similarity that distinguishes between homologs with divergent or similar functions. Using these methods Applicants identified a number of phylogenetically-related sequences that fall within the scope of the present claims, including soy sequence

SEQ ID NO: 969 (G3724), rice sequence SEQ ID NO: 971 (G3726), corn sequences SEQ ID NOs: 974 (G3804) and 975 (G3722). Applicants have also identified polypeptide sequences having conserved domains that are at least 55% identical with the conserved domain of G1274 (Exhibits A and B).

However, while a theoretical discussion such as that presented by Eisen (*supra*) provides expectations of functional relatedness between phylogenetically-related sequences, Applicants' evidence of possession of the invention and their understanding of the evolutionary and functional relationships between phylogenetically-related sequences is best exemplified by the data presented in the attached declaration by Dr. Peter Repetti. This declaration supports Applicants' identification of phylogenetically-related sequences from diverse species (dicots and monocots) that function in a manner similar to G1274 (SEQ ID NO: 194) by conferring greater tolerance to cold during germination, greater tolerance to cold during growth, greater tolerance to water deprivation, greater tolerance to nitrogen limitation, larger leaves, or greater biomass as compared to controls. These sequences were discovered using the phylogenetic analysis described in the specification to find G1274 orthologs, and, as shown in the attached declaration by Dr. Repetti, these phylogenetically-related sequences have conserved domains that are at least 55% identical to the conserved domain of the G1274 polypeptide. This supports present claims to polypeptides with conserved domains at least 70% identical to the conserved domain of G1274, and which function similarly. Percent identity and pairwise alignments were performed using BLASTp analysis as described in the specification (page 120, lines 9-14). Thus, by using phylogenetic analysis of the presently claimed sequences, the knowledge that conserved domains are correlated with conserved function, and the confirmation that sequences with conserved domains closely and phylogenetically-related to G1274 function similarly to G1274, Applicants successfully characterized the range of sequences encompassed by the presently claimed invention.

Regarding the teaching of a representative number of sequences, Table 7, page 185, rows 3-12 of the specification provides representative examples of closely-related sequences possessing conserved domains similar to that of G1274. Of these, soy sequence SEQ ID NO: 969 (G3724), rice sequence SEQ ID NO: 971 (G3726), corn sequences SEQ ID NOs: 974 (G3804) and 975 (G3722) have been shown to confer large size, water deprivation tolerance, cold tolerance, and/or low nitrogen tolerance, as does G1274, when these sequences are overexpressed in plants (see Exhibits A and B).

These sequences derive from diverse species and each is encompassed by the present claims. As those skilled in the art are aware, dicots and monocots from which these sequences derive are phylogenetically diverse, and thus the function of the SEQ ID NO: 969, 971, 974 and 975, and the sequences found in Exhibit B, has been highly conserved across a wide range of plant families, a clear and strong indication that this particular art of associating function with the claimed degree of sequence identity is predictable. These observations confirm that G1274 clade member sequences function similarly to G1274.

Thus, Applicants believe the recitation of University of California v. Eli Lilly does not pertain to the present claims. In Lilly at 1567, the cDNA for human insulin had never been characterized. The present claims are directed to sequences described by much more than their biological function alone, including the specific structural feature of the conserved domain of G1274, common to and functional in sequences encompassed by the claims. By identifying sequences from diverse species (thus representing a substantial portion of the claimed genus) in the specification and in the attached Exhibits and Declaration, Applicants have demonstrated that the claimed structural features, conserved domains exemplified by the conserved domain of G1274, are correlated with greater tolerance to cold during germination, greater tolerance to cold during growth, greater tolerance to water deprivation, greater tolerance to nitrogen limitation, larger leaves, or greater biomass.

In light of these amendments, arguments and experimental observations confirming Applicants' disclosure and claims, Applicants request that the rejection under 35 U.S.C. §112, first paragraph, written description, be withdrawn.

Office action, item 5, rejection under 35 U.S.C. §112, first paragraph, enablement

The rejection for lack of enablement is respectfully traversed for the following reasons.

The Examiner has stated that the present application contains subject matter which was not described in such a way as to enable one skilled in the art to which it pertains to make and or use the invention. In his arguments, the Examiner has referred to the Wands factors for indicating the degree of experimentation that would be necessary to practice the invention.

Regarding the amount of direction or guidance presented in the specification, the state of the prior art, and the relative skill of those in the art, Applicants noted above that conserved domains and functions conserved between phylogenetically-related sequences are well known in the art.

Regarding the breadth of the present claims, Applicants have provided experimental evidence (see declaration by Dr. Repetti) indicating that the present scope is supported by the specification as filed and properly dictated by the phylogenetic relatedness of the claimed sequences.

Regarding the predictability of the art and the presence or absence of working examples, sequences that fall within the scope of the present claims have been used to make overexpressing lines that have greater tolerance to cold during germination, greater tolerance to cold during growth, greater tolerance to water deprivation, greater tolerance to nitrogen limitation, larger leaves, and greater biomass than wild-type plants. Sequences that fall within the scope of the claims and which have been tested in plants conferred these traits when overexpressed in plants, even though in some cases, only a few lines of plants were examined. These sequences derive from *Arabidopsis* and soy (dicots) as well as corn and rice (monocots). Dicots and monocots are phylogenetically diverse, and since the function of these sequences has been highly conserved across distantly-related plant families, rather than undue experimentation, it would be a matter of routine for the

skilled artisan to practice the invention by transforming plants and expressing within them orthologous polypeptide sequences that have conserved domains that are at least 70% identical to the G1274 polypeptide, SEQ ID NO: 194. Applicants listed soy sequence SEQ ID NO: 969 (G3724), rice sequence SEQ ID NO: 971 (G3726), and corn sequences SEQ ID NOs: 974 (G3804) and 975 (G3722) in their specification and identified these sequences as orthologs (e.g., in "Table 7. Orthologs of Representative *Arabidopsis* Transcription Factor Genes", in which these SEQ ID NOs. appear on page 185, in "Table 9. Similarity relationships found within the Sequence Listing", in which these SEQ ID NOs. appear on page 337, and in the Sequence Listing as SEQ ID NOs: 969, 971, 974, and 975). These sequences have been shown to confer large size, water deprivation tolerance, cold tolerance, and/or low nitrogen tolerance, as does G1274, when these sequences are overexpressed in plants (see Exhibits A and B). Thus, Applicants believed at the time the present application was filed that the art of predicting functions of closely, phylogenetically-related sequences was predictable, as indicated by Eisen (*supra*), and have so demonstrated the breadth of the present claims.

Applicants indicated in their specification that "[t]ranscription factors that are homologous to the listed sequences will typically share, in at least one conserved domain, at least about 70% amino acid sequence identity"(page 39, lines 1-2) and "[a]nalysis of groups of similar genes with similar function that fall within one clade can yield sub-sequences that are particular to the clade" (page 36, lines 27-28). In an alternative viewpoint, the Examiner has stated that the "state of the art is such that one of skill in the art cannot predict which nucleic acids that are 70% sequence identical to amino acid coordinates 111-164 of SEQ ID NO: 194 will encode a protein with the same activity as amino acid coordinates 111-164 of SEQ ID NO: 194. In fact, one can (and Applicants did) predict polypeptide activity for related sequences provided a phylogenetic-based approach such as that described by Eisen (*supra*), or provided by Applicants, is used to identify sequences that derive from a common ancestral sequence and lie within a closely-related clade (see page 36, line 17 through page 37, line 7 of the present specification).

The example of Bowie et al. cited by the Examiner, indicates that proteins are sensitive to alterations of even a single base pertains to sequences that may or may not be naturally selected to retain function. As noted above, functional predictions can be greatly improved by focusing on how the genes became similar in sequence (i.e., by evolutionary processes) rather than on the sequence similarity, or in this case, dissimilarity, itself (Eisen, *supra*). In fact, on pages 1306-1307, Bowie cites a study of sequence tolerance in which the "substitutions were identified by a *functional* selection after cassette mutagenesis" (*emphasis* added). In contrast, orthology or phylogenetic-relatedness infers strong selection to *retain* a particular function. Bowie et al. considered "allowed amino acid substitutions" or "functionally conserved residues" that "should be conserved in sets of active sequences". However, Bowie et al. do not address the likelihood that residues will be conserved in phylogenetically-related proteins falling within a closely-related clade. As those skilled in the art are aware, transcription factors have roles in regulating transcription of at least one and often numerous

functions within an organism, and there is strong selective pressure to retain function in closely-related plant species. In other words, unless one is trying to make a protein non-functional or attacking particular residues in a random manner, proteins closely-related to G1274 should retain similar function and would be provide a plethora of functional sequences that are routine to discover. Rather than an approach that first identifies sequence variations and then examines whether a function is lost or retained, a phylogenetic approach assumes similar functions are retained after speciation and should thus be used to ferret out related sequences with evolutionarily-conserved functions. The present specification and the evidence provided in the declaration by Dr. Repetti precisely and clearly confirm this analysis. Since all of the sequences that have been tested (sometimes with just a few lines) with at least 55% identity to the conserved domain of G1274 confer at least some, and often, many of the same traits as does G1274, one skilled in the art would recognize that sequences encompassed by the present claims would be more likely to represent operable rather than inoperable species.

Regarding the McConnell reference, the fact that a single mutation CAN result in an alteration in transformation of abaxial leaf fate is not pertinent to the present specification. As noted above, functional predictions can be greatly improved by focusing on how the genes became similar in sequence (i.e., by evolutionary processes) rather than on the sequence similarity itself (Eisen, *supra*). Clearly, the glycine to glutamic acid substitution taught in this reference is important in determining leaf polarity, but what is not clear from this reference is whether this substitution invalidates the supposition that many sequences are likely to retain similar function. Of course there are means to destroy the function of virtually any protein, even without altering a particular sequence; one could denature it, fuse a long, inert sequence to one or both termini, or wrap it in plastic. Whether a protein can have its function dramatically altered by a substitution in a surface or conformation-critical residue is not the issue; the issue is whether organisms that need a particular function are likely to retain that function by retaining similarly in their ancestrally-derived proteins that mediate that function, and whether this provides a readily available supply of sequences of the invention that may be routinely discovered by the skilled artisan. The evidence in the art and presently provided strongly indicate that this is the case.

The Miao reference cited by the Examiner teaches a "pull down assay" to identify target sequences of WRKY53 (a protein in the same family but distantly related to G1274). This reference attempts to clarify relationships with a specific transcription factor signaling cascade. The reference does not describe or imply how sequences closely-related to WRKY53 might function or not function in the same or similar fashion. The observation in plants overexpressing WRKY53 that, "five other member of the WRKY protein family were strongly expressed, but were not detectable in the respective wild-type plants" does not seem to be relevant to the present rejection. The fact that five WRKY proteins are apparently involved in a specific

cascade does not preclude a sequence closely-related to WRKY53 from initiating the same or a similar cascade and thus conferring same or similar functions.

With regard to the Examiner's contention that Applicants have not disclosed how to make or isolate any of the sequences encompassed by "Applicants broad claims", this aspect of the rejection is addressed above in the argument that describes the phylogenetic approach to sequence identification and analysis. Certainly, SEQ ID NOs: 969, 971, 974, and 975 from very diverse species were disclosed, and those do function as claimed. Additional sequences that fall within the scope of the claims and that appear in Exhibits A, B and D also confer the presently claimed traits. Thus, Applicants provided a functional sequence in the form of G1274 and methods for identifying and confirming function of closely-related sequences, some of which were provided in Table 7. Applicants identified a conserved domain (a structure of the sequence associated with function). Applicants also point out that Examples I through V (page 361, line 16 through page 364, line 22) disclose methods for full length gene identification and cloning, transforming *Agrobacterium*, transforming *Arabidopsis* and other plants with *Agrobacterium tumefaciens*, and identifying primary transformants and modified phenotypes. Methods for isolating transcription factor-encoding cDNA using PCR and primers designed from a presently disclosed transcription factor gene sequence are described in the specification (for example, on page 41, line 28 through page 42, line 2, and in Examples I and II). Furthermore, methods for making or isolating polynucleotide sequences are well known in the art and are routinely performed by technical assistants. A patent specification need not teach, and preferably omits, what is well known in the art. Hybritech Inc. v. Monoclonal Antibodies, Inc., 802 F.2d 1367, 1384, 231 USPQ 81, 94 (Fed. Cir. 1986). In fact, recombinant DNA technology, including amplification and primer design, is now so routine it may be found in high school curricula. See, for example, Kreuzer and Massey (2000) "Recombinant DNA and Biotechnology : A Guide for Teachers", ASM Press, ISBN: 1555811752, page 187-190 http://www.amazon.com/gp/reader/1555811760/ref=sib_dp_bod_toc/002-8434597-0474417?%5Fencoding=UTF8&p=S002#reader-link

Regarding the "undue trial and error experimentation" which "would be required for one of ordinary skill in the art to screen through the multitude of non-exemplified sequences", Applicants note that all of the sequences closely-related to SEQ ID NO: 194 that fall within the scope of the present claims, that are derived from evolutionarily diverse species, and that have been transformed into plants can confer similar functions when overexpressed, as confirmed in the declaration of Dr. Repetti. A few of the sequences lying just outside the clade (having descended from a common but somewhat more distant ancestor) have diverged somewhat further from G1274 than G1274 clade members, but even some of these have retained functions found in the present claims. These latter sequences help identify the limits of the G1274 clade boundary and the scope of the present claims.

Thus, just as Applicants have done, it would be a matter of routine to identify sequences that fall within the G1274 clade and the scope of the present claims, and to identify closely-related sequences with similar activity to G1274.

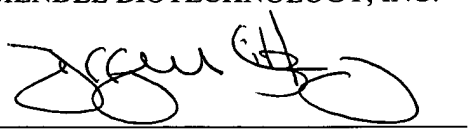
The rejection of Claim 30 is avoided by the amendment canceling Claim 30.

In light of these amendments, arguments and experimental observations confirming Applicants' disclosure and claims, Applicants request that the rejection under 35 U.S.C. §112, first paragraph, for lack of enablement, be withdrawn.

CONCLUSION

Applicants believe that no additional fee is due with this communication. However, if the USPTO determines that a fee is due, the Commissioner is hereby authorized to charge Mendel Biotechnology, Inc. Deposit Account No. **50-1025.**

Respectfully submitted,
MENDEL BIOTECHNOLOGY, INC.



Date: February 13, 2006

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Attachments:

- Declaration under 37 CFR 1.132 OF Peter Repetti
- Exhibit A
- Exhibit B
- Exhibit C
- Exhibit D
- Reference: Li and Herskowitz
- Reference: Jaglo
- Reference: Eisen
- Reference: Hurley
- Reference: Marchler-Bauer

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